AD 424189

SAM-TDR-63-75

STUDIES WITH A SIMULATED MARTIAN ENVIRONMENT

Germination and Growth of Bacterial Spores

TECHNICAL DOCUMENTARY REPORT NO. SAM-TDR-63-75

September 1963

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

Task No. 775302

FOREWORD

This report was prepared by the following personnel in the Astrobiology Section:

THOMAS L. ROBERTS, M.S.

LAURENCE A. IRVINE, Captain, USAF, MSC

STUDIES WITH A SIMULATED MARTIAN ENVIRONMENT
Germination and Growth of Bacterial Spores

1. INTRODUCTION

Many reports have appeared in the literature concerning survival and limited multiplication of bacteria in a simulated Martian environment. Kooistra et al. (5) reported survival and multiplication of bacteria in a simulated Martian environment containing an atmosphere of nitrogen only. Their results were confirmed by Davis and Fulton (2) with a strain of *Bacillus cereus*. Hawrylewicz et al. (4) reported that *Clostridium botulinum* spores and vegetative cells of *Bacillus subtilis* survive, with the latter showing growth, under a simulated Martian environment. *Escherichia coli* showed rapid decrease in viability but survived this environment. Other reports (3, 8) have appeared, indicating that various bacteria are able to survive and multiply. Roberts and Wynne (9) reported statistically significant increases in colony counts of *B. cereus*. They also suggested (10) that because of temperature cycling, dormancy was interrupted and germination of spores increased the colony counts.

In the reports cited, a number of bacterial species were exposed in containers in which Martian conditions were simulated according to the best data available. These environments included a minimum of water (1% or less). In general, these experiments did not offer possibilities for the practical use of microorganisms in this environment. The lack of conclusive evidence for the existence of water on Mars has resulted in a general belief that water, if present at all, is in a frozen or a sublimated state. Several reports (1, 6, 7) have indicated that a possible Martian "hydrosphere" in warm seasons may produce an abundant water supply due to thawing of Martian polar caps. It has been suggested that much of this moisture may be concentrated in the soil in limited areas (6). If such moisture were present, it could provide an area suitable for the growth of plants or other organisms.

Because our previous experiments considered only the environmental conditions for the entire planet and failed to consider the possibility that ground water might be localized, we have evaluated germination of spores and growth of bacteria in a simulated Martian microenvironment with added moisture.

Received for publication on 1 July 1963.

2. SUMMARY

The results presented show that *Clostridium sporogenes* spores germinate and multiply in a simulated Martian environment containing 8% soil moisture. These findings corroborate previous reports (2, 9) of increased colony counts of sporeformers in a simulated Martian environment. Present findings indicate that the amount of moisture required for germination and growth is a more critical factor than the proposed temperature or gaseous composition.

3. MATERIAL AND METHODS

Clostridium sporogenes (PA 3679) spores grown in brain-heart infusion broth at 37° C. for 2 weeks were harvested, washed, and stored in water at 2° C. for approximately 6 months. Prior to use, the suspension was heated at 60° C. for 30 minutes, washed, and finally resuspended in 10 ml. of distilled water. Bacteriologic staining indicated no debris or contamination of the final suspension. Aliquot portions (1 ml.) of this spore suspensión were placed on sterile glass culture slides.

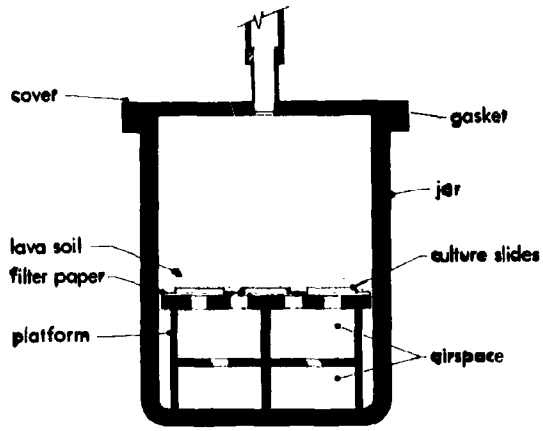

FIGURE 1

Soil chamber used to simulate a Martian micro-environment.

Enriched soil was prepared by washing 500 gm. of pulverized red lava with 1 liter of distilled water and concentrating the supernatant fluid to 250 ml. by evaporation. This fluid was then used to suspend 150 gm. of lava which had been previously pulverized and passed through a 50-mesh stainless steel wire grating. The wet soil was dried and twice sterilized by autoclaving. Sterility tests showed no viable organisms.

For direct visualization, eight "soil chambers" were constructed by placing the spore suspension culture slides on filter paper on a plastic platform in jars previously sterilized with ethylene oxide, and pressing 75 gm. of the enriched pulverized lava upon three of the moist culture slides. The layer of lava was about 0.5 to 1 cm. in depth and 12 cm. in diameter. Water (5 ml.) was placed at each of four points and absorbed into the soil (fig. 1). The chamber was then closed.

For quantitative counts, the remaining spore suspension was mixed with 40 gm. of the crushed lava and 1.12 gm. aliquot portions of the mixture were placed in 2 ml. glass tubes. Six of these tubes were placed in each of eight jars designated "Mars jars." Distilled water (0.5 ml.) was added to each tube.

A simulated Martian environment was established in the Mars and culture chambers by evacuating to 4 mm. Hg. They were then

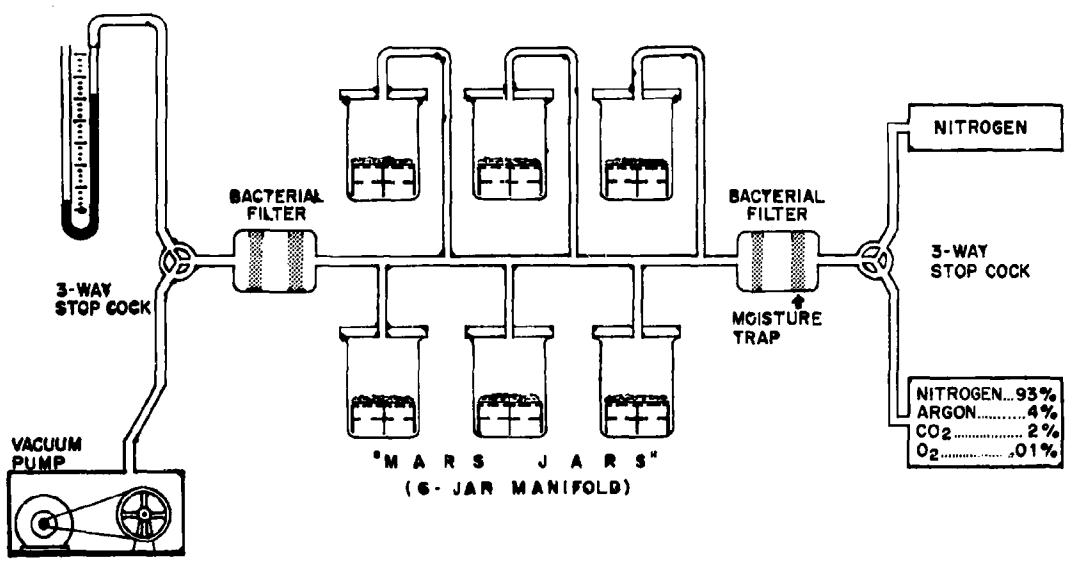

FIGURE 2

System used to establish simulated Martian atmospheric conditions.

flushed four times with moisture-free nitrogen and filled with a gaseous mixture containing 94% nitrogen, 4% argon, and 2% carbon dioxide to a pressure of 65 mm. Hg. The mixture contained less than 0.01% oxygen (fig. 2). Temperature and light were regulated to give a nocturnal-diurnal cycle of 8 hours of darkness at temperatures ranging from 23° to −25° C. followed by 16 hours of light at temperatures ranging from −25° to 23° C. (fig. 3). Four jars serving as controls were run in room atmosphere at 23° C.

On days 0, 7, 14, and 28, one Martian and one control jar were entered, and three glass tubes were removed from each for bacterial counts and three for moisture determination. After examination these jars were discarded. Before entry, the internal pressure was verified to be approximately 65 mm. Hg. Triplicate platings were made from serial dilutions of each of the glass tubes for bacterial counts in a medium of liquefied yeast extract, thioglycollate supplement, starch, K_2HPO_4, and agar (11). Tubes of the medium were kept in a water bath (45° C.) until inoculated with 1 ml. of appropriate dilutions. After the medium was inoculated, poured, and allowed to solidify, an anaerobic seal was provided by overlaying the surface with thioglycollate supplement agar. Plates were incubated for 2 days at 37° C.

Moisture determinations were made on aliquot portions of soil in moisture-free 7 ml. screw-cap vials. After weighings, the vials were dried at 105° C. for 48 hours, reweighed, emptied of soil, and weighed again.

On days 7, 14, and 28, spore suspension culture slides were removed from each of the "soil chambers" and the soil was removed from the slides. Preparations were fixed, washed gently in cold distilled water to remove fine soil particles, and stained. The slides were examined under the microscope, and photomicrographs were made.

4. RESULTS AND DISCUSSION

In table I each figure for colony counts is the average from triplicate platings of appropriate 10-fold serial dilutions of inoculated soil specimens taken from each of three glass

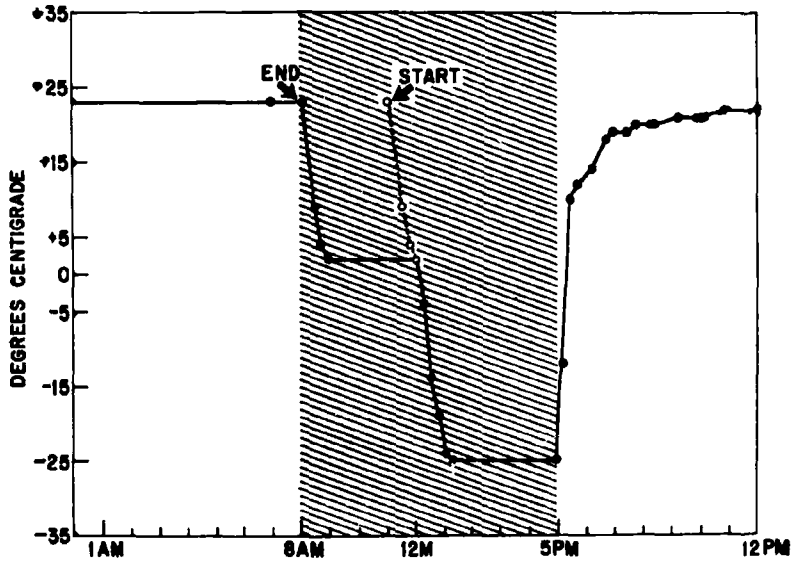

FIGURE 3

Nocturnal-diurnal temperature cycle; 8 hours' darkness (hatched area) and 16 hours' light.

TABLE I

Response of Clostridium sporogenes *in tubes when exposed to a simulated Martian microenvironment (Mars jars)*

Days	Controls		Microenvironment	
	Colony counts	Percent moisture	Colony counts	Percent moisture
0	1.7×10^6	8.7	1.7×10^6	8.3
7	3.7×10^6	8.0	2.9×10^6	8.1
14	2.8×10^7	8.5	4.0×10^6	8.8
28	3.7×10^8	8.6	4.2×10^7	8.4

tubes. Each figure for percent moisture is the average of three soil specimens used for analysis. Both test specimens and controls showed increases in colony counts with time (fig. 4) and no significant variations in soil moisture. Percent moisture values of nine random specimens from the "soil chambers" averaged 11% with extremes of 8.4% and 14.8%.

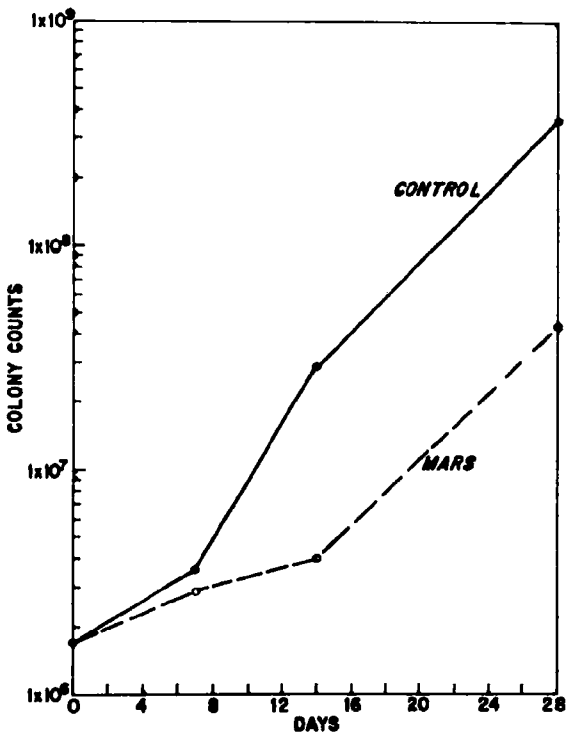

FIGURE 4

The effect of simulated Martian environment on growth of Cl. sporogenes.

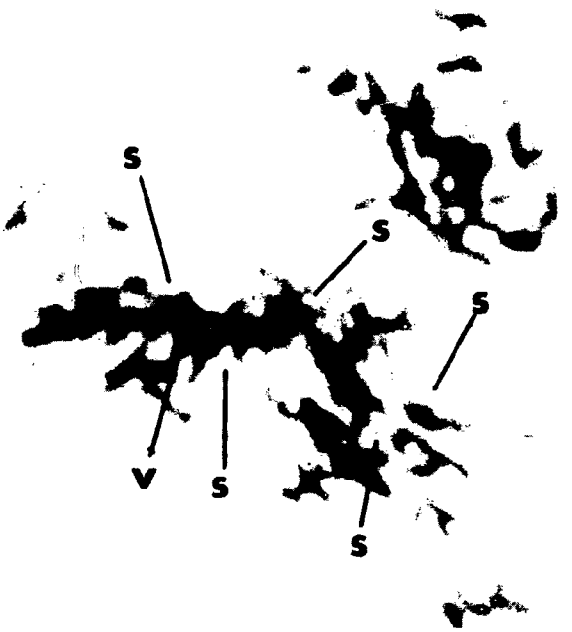

FIGURE 5

Culture slide 7 days in simulated Martian environment. S, *spore structures;* V, *vegetative cells.*

When the control spore suspension culture slides were stained by Gram's method and with carbolfuchsin–methylene blue stain, and examined under a light microscope, only structures resembling spores were found. After 7 and 14 days in the "soil chambers," slides examined by this method showed that both gram-positive vegetative cells and spores were present, the vegetative cells predominating (figs. 5 and 6). At 30 days only vegetative cells were present (fig. 7). Colony counts confirmed visual indication of an increase in number of vegetative cells. The increase was somewhat greater and more rapid in the control "Mars jars" at 23° C. than in the more rigorous conditions of the Martian temperature cycling jars.

Incubation of *Cl. sporogenes* spores in the "soil chambers" simulating a Martian microenvironment with added moisture also resulted in germination and multiplication of these

bacterial specimens. These findings do not indicate that temperature cycling is *the* important factor as was suggested by Davis and Fulton (2); it appears, rather, that the amount of moisture is a more critical factor. The presence of carbohydrates and proteins in the test soil—not considered an important factor in previous reports—may greatly influence multiplication of the bacteria. This species of bacteria produces extracellular proteolytic enzymes (proteinases and peptidases) that convert protein present in the soil to peptides and amino acid nutrients.

FIGURE 6

Culture slide 14 days in simulated Martian environment.

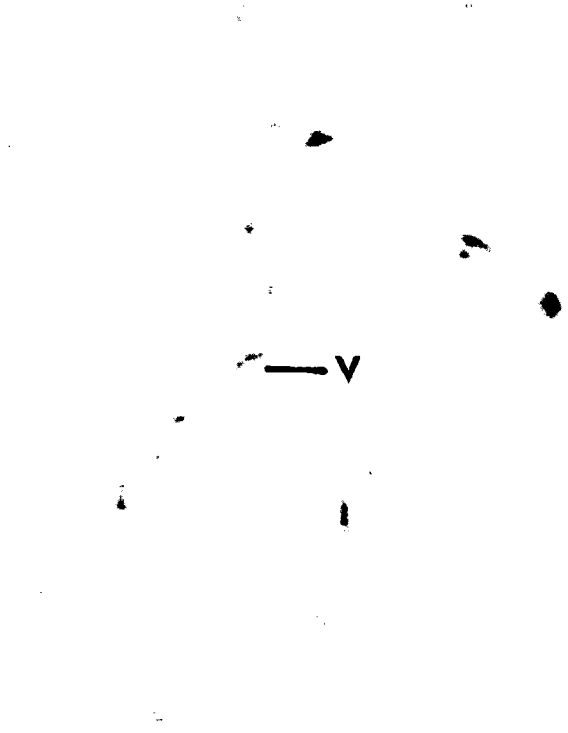

FIGURE 7

Culture slide 30 days in simulated Martian environment.

The transition of a bacterial spore culture to a growing vegetative cell culture in a simulated Martian environment is not a simple abrupt reaction, nor does it depend primarily on temperature or moisture alone. Growth at 23° C. compared with that at cycled Martian temperatures is not significantly different. The comparison indicates that the most important *single* requirement for growth of terrestrial organisms is water and that the presently used temperatures and atmospheres play a minor part in limiting such growth. We, therefore, question the value of experiments with simulated planetary environments that exclude water.

USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-75. STUDIES WITH A SIMULATED MARTIAN ENVIRONMENT. GERMINATION AND GROWTH OF BACTERIAL SPORES. Sept. 63, 6 pp. incl illus, table, 11 refs.

Unclassified Report

Simulated Martian microenvironment containing 8% moisture permitted germination and growth of endospores of *Clostridium sporogenes*.

1. Microorganisms on Mars
2. Extraterrestrial life

I. AFSC Task No. 775302
II. T. L. Roberts; L. A. Irvine, Capt., USAF, MSC
III. In DDC collection

USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-75. STUDIES WITH A SIMULATED MARTIAN ENVIRONMENT. GERMINATION AND GROWTH OF BACTERIAL SPORES. Sept. 63, 6 pp. incl illus, table, 11 refs.

Unclassified Report

Simulated Martian microenvironment containing 8% moisture permitted germination and growth of endospores of *Clostridium sporogenes*.

1. Microorganisms on Mars
2. Extraterrestrial life

I. AFSC Task No. 775302
II. T. L. Roberts; L. A. Irvine, Capt., USAF, MSC
III. In DDC collection

USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-75. STUDIES WITH A SIMULATED MARTIAN ENVIRONMENT. GERMINATION AND GROWTH OF BACTERIAL SPORES. Sept. 63, 6 pp. incl illus, table, 11 refs.

Unclassified Report

Simulated Martian microenvironment containing 8% moisture permitted germination and growth of endospores of *Clostridium sporogenes*.

1. Microorganisms on Mars
2. Extraterrestrial life

I. AFSC Task No. 775302
II. T. L. Roberts; L. A. Irvine, Capt., USAF, MSC
III. In DDC collection

USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-75. STUDIES WITH A SIMULATED MARTIAN ENVIRONMENT. GERMINATION AND GROWTH OF BACTERIAL SPORES. Sept. 63, 6 pp. incl illus, table, 11 refs.

Unclassified Report

Simulated Martian microenvironment containing 8% moisture permitted germination and growth of endospores of *Clostridium sporogenes*.

1. Microorganisms on Mars
2. Extraterrestrial life

I. AFSC Task No. 775302
II. T. L. Roberts; L. A. Irvine, Capt., USAF, MSC
III. In DDC collection

UNCLASSIFIED